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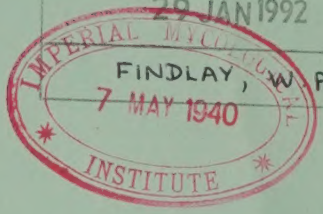

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Findlay & Pettifer

RESEARCH NOTES

EFFECT OF BLUE STAIN ON THE STRENGTH OF OBECHE (*TRIPLOCHITON SCLEROXYLON*)

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(1) Introduction.

OBECHE logs are very liable to develop fungal stain unless they are converted soon after felling. Usually this timber is shipped from Nigeria or the Gold Coast in the form of large squared logs, and if these have been left lying about in the forest or in storage for any length of time before shipment they may arrive in Europe in a badly stained condition and even contain incipient decay. The fungus which has been isolated most frequently from the blue stained areas in these logs is *Botryodiplodia theobromae* Pat. This fungus is known as a frequent cause of die-back and pod-rot in cocoa. It is very widely distributed and common on a wide range of vegetable material.

Previous work by the authors (1) has shown that blue stain caused by *Ophiostoma* (*Ceratostomella*) sp. brings about an appreciable reduction in toughness, but scarcely affects the other strength properties of pine sapwood.

Since the fungi which cause staining in the light coloured tropical hardwoods such as Celtis and Obeche are usually of a somewhat different type from those which cause blue stain in pine, it was, therefore, considered important to determine whether this "*Diplodia*" stain has any serious effect on the strength of a timber such as Obeche which is quite important economically.

(2) Material for Test.

A freshly felled log of Obeche, about 6 feet long and 30 inches diameter, was obtained in January 1939 through the courtesy of the United Africa Company. As the sapwood is more susceptible to staining than the heartwood, only the outer part of the log was used for these tests, planks about $1\frac{1}{8}$ inches thick, from which the test pieces were subsequently obtained, being cut from the periphery.

(3) Strength Properties Investigated and Test Pieces Used.

The strength properties investigated were bending strength (equivalent fibre stress at maximum load), stiffness (modulus of elasticity) and toughness

(energy absorbed in fracture), and in addition the nominal specific gravity* was determined.

The bending strength and stiffness were determined from test pieces 5 inches long and $\frac{3}{8}$ inch square in cross section. This small size was used to facilitate the uniform infection and staining of the specimens. Toughness was determined from the standard size test piece 10 inches long and $\frac{5}{8}$ inch square. All the specimens were selected so that they were straight-grained and free from knots, checks or other defects.

(4) Matching of Test Specimens.

Previous work had shown that the extent of staining of the individual members of a group of test pieces exposed for a given time, under similar conditions, to the action of a staining fungus, varied very considerably, some pieces being very heavily stained over the whole of the cross section, while others were only very lightly stained. Since the degree of staining did not appear to be very closely related to the time of exposure to the fungus, it was arranged to match the specimens individually with their own controls rather than in groups with groups of controls. The test pieces were accordingly cut in pairs side by side and as nearly as possible from the same growth rings, one of each pair being tested as control and the other being tested after exposure to the staining fungus.

In previous experiments it had been shown that the staining fungus caused a greater reduction in strength of wood which had previously been sterilized by intermittent steaming than in the strength of unsterilized wood, therefore the effects of staining on the mechanical properties of both unsterilized wood and sterilized wood were investigated, the control test pieces for the latter being, of course, also sterilized prior to test. In addition, the effects of steam sterilization, *per se*, on the properties of the timber were determined from matched pairs, one member of which was unsterilized and the other steamed.

In all, 200 test pieces for static bending tests and the same number for toughness tests were prepared, the allocation given below being the same for each of these two tests, Groups 1 and 2 being tested after different periods of exposure to the staining fungus :

	Number of Specimens	
	Stained	*Control*
Unsterilized, stained—Group 1	20	20
Group 2	20	20
Sterilized, stained —Group 1	20	20
Group 2	20	20
Sterilized, unstained	20	
Control	20	

(*) The term "nominal specific gravity" is defined in British Standard, No. 373—1938, Methods of Testing Small Clear Specimens of Timber, as "the ratio of the weight of wood substance non-volatile at 100° C. to the weight of a volume of water equal to that of the specimen at time of test". The weight of wood substance non-volatile at 100° C. is the oven-dry weight given in previous definitions of nominal specific gravity.

FIGURE I. Radial section of Obeche with dark coloured hyphae of *Botryodiplodia theobromae* in the medullary ray cells. x 360.

FIGURE II. Longitudinal section of Obeche showing dark coloured hyphae of *Botryodiplodia theobromae* in the wood parenchyma. Cell walls stained with safranin. x 360.

(5) Sterilization, Infection and Incubation.

One-half of the test pieces were sterilized by steaming at atmospheric pressure for forty-five minutes on each of three successive days. The rest which hereafter are referred to as "unsterilized" were exposed for about one minute to a jet of live steam in order to reduce surface contamination with foreign organisms.

The small static bending test pieces were infected by being placed on actively growing cultures of the test fungus in special Kolle flasks. The fungus was grown on a medium consisting of 2 per cent malt extract, 1 per cent starch and 2 per cent agar. The toughness test pieces were infected by placing them on sloped cultures of the fungus on the same medium, in test tubes, 18 by 3 inches in size.

The flasks and tubes containing the test pieces were kept in an incubator maintained at 22°C., Group 1 being removed after six and Group 2 after twelve weeks' exposure to the fungus.

(6) Methods of Test.

The control test pieces were all tested at the beginning of the experiment, those for comparison with the steamed material being tested immediately after sterilization.

At the end of the given period of incubation, the test pieces were removed from the flasks, and after having been wiped carefully to remove superficial mycelium they were weighed and measured.

The test pieces for the static bending test were supported freely over a span of 4.67 inches and loaded centrally, deflection readings being taken at appropriate load increments up to the maximum load. The modulus of elasticity was obtained from the load-deflection graph and the equivalent fibre stress at maximum load was computed from the usual formula. After test, the whole specimen was oven dried for estimation of its moisture content.

The toughness test pieces, freely supported over a span of 8 inches were broken in a machine of the pendulum type by a single blow, centrally applied. The moisture content of the specimens, tested after six weeks' incubation, which was used in the determination of the nominal specific gravity, was estimated from a sample about 4 inches long cut from one end. The values so obtained were found to be erratic, owing to the uneven distribution of moisture over the specimens as a result of the conditions of incubation. For the remaining group, tested after twelve weeks' incubation, the whole test piece was dried to obtain a more representative average figure.

(7) Results of Tests.

(*a*) *Effects of Steam Sterilization.*

The results of tests to determine the effect of steam sterilization alone on the strength of obeche are given in Table I.

TABLE I.

Effect of Steaming on the Strength of Obeche.

Properties.	Steamed.	Control.
Static Bending :		
Number of results	20	20
Nominal specific gravity	0.34	0.33
Equivalent Fibre stress at maximum load : lb./sq. in. ..	6810	7390
Modulus of elasticity : 1000 lb./sq. in.	680	700
Toughness :		
Number of results	19	19
Nominal specific gravity	0.36	0.35
Energy absorbed—inch/lb.	137	140

(*b*) *Effects of Blue Stain.*

The results of the tests on the stained material after six weeks' and after twelve weeks' exposure to the fungus are given in Table II.

A visual estimate was made of the percentage of staining over a cross section taken as near to the fracture as feasible and an attempt was made, without success however, to correlate this percentage with the percentage decrease in strength. The erratic nature of the results may have been due, in part at any rate, to the margin of error associated with the visual estimation of the staining and in part, to the difficulty of ensuring perfectly matched specimens for controls. The test pieces were therefore divided into two groups, those with a low degree of staining, having 25 per cent or less of the cross section showing blue stain, and those with a moderate to high degree of staining, showing 25 per cent or more. Table II gives the results classified on this basis, together with figures for all the stained samples, irrespective of the degree of staining. The mean values of the matched controls for each of the groups are also included in the table, together with figures for the percentage decreases in strength and specific gravity due to staining.

MYCOLOGICAL EXAMINATION.

Sections were cut from the test pieces as close as possible to the point of fracture and examined under the microscope. Some sections were mounted without staining, others were stained in safranin and picro-aniline blue. The fungus has conspicuous thick dark brown hyphae which are abundantly septate and occasionally bear little outgrowths that superficially resemble clamp connexions (*see* Figs. 1 and 2). The hyphae are present in the rays and wood parenchyma and occasionally masses of mycelium filling some of the larger vessels may be found. Very rarely were the hyphae seen in the fibres. At the stage of attack examined, penetration of the cell walls seemed to be almost entirely by means of the pits and very few cases of direct penetration of the walls were observed.

Table II.

Effect of Blue Stain on Mechanical and Physical Properties of Obeche.

Treatment	Period of Incubation Weeks	Degree of Staining	STATIC BENDING TEST									TOUGHNESS TEST					
			Number of Results	Nominal Specific Gravity			Equivalent Fibre Stress at Max. Load—lb./sq. in.			Modulus of Elasticity 1000 lb./sq. in.			Number of Results	Nominal Specific Gravity			Energy Absorbed inch/lb.		
				Mean Values		*Per Cent Decrease	Mean Values		*Per Cent Decrease	Mean Values		*Per Cent Decrease		Mean Values		*Per Cent Decrease	Mean Values		*Per Cent Decrease
				Stained	Control		Stained	Control		Stained	Control			Stained	Control		Stained	Control	
Unsterilized Stained	6	Low	2	0·31	0·32	3·2	6900	7430	6·5	720	740	1·8	6	0·32	0·35	8·8	121	144	16·6
		Moderate and High ..	18	0·32	0·35	7·7	6540	7510	12·7	670	740	10·0	14	0·33	0·34	1·8	101	130	22·9
		Low, Moderate and High ..	20	0·32	0·34	7·2	6570	7510	12·1	670	740	9·2	20	0·33	0·34	3·9	107	134	21·0
	12	Low	—	—	—	—	—	—	—	—	—	—	10	0·33	0·35	6·1	115	151	24·7
		Moderate and High ..	20	0·31	0·34	8·9	6100	7650	19·7	680	720	5·1	10	0·32	0·35	9·1	82	145	43·3
		Low, Moderate and High ..	20	0·31	0·34	8·9	6100	7650	19·7	680	720	5·1	20	0·32	0·35	7·6	99	148	34·0
Sterilized Stained	6	Low	4	0·30	0·33	8·1	6270	6400	1·0	620	690	9·2	6	0·31	0·35	11·8	103	129	19·5
		Moderate and High ..	16	0·32	0·35	7·3	6370	6820	6·0	680	680	0·2	14	0·31	0·36	12·9	78	132	39·8
		Low, Moderate and High ..	20	0·32	0·34	7·5	6350	6740	5·0	670	680	2·0	20	0·31	0·36	12·5	85	131	33·7
	12	Low	—	—	—	—	—	—	—	—	—	—	9	0·32	0·34	7·3	119	148	19·7
		Moderate and High ..	20	0·31	0·34	7·5	6050	6910	11·6	690	720	3·3	11	0·30	0·35	12·3	94	131	29·3
		Low, Moderate and High ..	20	0·31	0·34	7·5	6050	6910	11·6	690	720	3·3	20	0·31	0·35	10·1	105	139	25·0

* The percentage decrease, quoted in these columns, is the average of the decreases for the individual matched pairs of control and stained specimens, and not the decrease obtained from the average figures quoted above for stained and control specimens.

In general the amount of mycelium observed under the microscope agreed closely with the depth of the visible grey blue staining. Some of the test pieces were naturally a rather darker yellowish-brown than the others, and this made observations on the depth of fungal staining more difficult. This brown colour was seen on microscopic examination to be due to the presence, mainly in the rays, of a yellowish-brown material.

Discussion of Results.

(*a*) *Effects of Steam Sterilization Alone.*

The slight apparent increase in nominal specific gravity of the steamed material compared with the control was found to be statistically insignificant, that is, it might have been due to the variability in the material alone. This finding is in agreement with the results of similar tests on beech (as yet unpublished) and Scots pine (1), in which it was found that steaming alone, as might be expected, produced no change in this property.

The decrease in equivalent fibre stress at maximum load after steaming was found to be statistically significant. In the tests on beech and Scots pine referred to above, no determinations were made of this property, but tests on compressive strength parallel to the grain, a closely related property, also showed a significant decrease due to steaming.

The decreases in modulus of elasticity and in toughness were found to be statistically insignificant, although in the tests on beech referred to above, a significant increase in toughness was found to accompany steaming.

Thus, these tests showed that for Obeche, nominal specific gravity, modulus of elasticity and toughness were all unaffected by steaming, but that the equivalent fibre stress at maximum load was decreased by some 8 per cent. The conclusions reached for these specific tests were confirmed by the data for the steamed and the unsterilized controls given in Table II. (*Note :* The toughness control value for the twelve weeks' unsterilized stained group is appreciably higher than that for any of the remaining groups, but the mean value of the combined controls for the unsterilized material—141 inch lb.—was found not to differ significantly from the mean value of the combined controls for the sterilized material—135 inch lb.).

(*b*) *Effects of Staining.*

(1) *Specific Gravity.* Reference to Table II shows that staining was accompanied by a decrease in the value of specific gravity, and it was found that the decrease of 7.2 per cent in this property, obtained for the static bending tests on unsterilized stained material after six weeks' incubation, was statistically significant. After twelve weeks the unsterilized toughness samples had lost 7.6 per cent of their over dry weight, while the toughness samples which had been steam sterilized before infection lost 12.5 per cent after only six weeks' exposure. Since these figures are greater than could be accounted for by attack on the cell contents alone, a chemical analysis of the heavily stained samples was undertaken (see below).

In the tests on Scots pine referred to above, it was also found that a significant decrease in specific gravity, though of a smaller order than that obtained for Obeche, accompanied the staining.

(2) *Equivalent Fibre Stress at Maximum Load.* A definite decrease in bending strength (equivalent fibre stress at maximum load) accompanied the staining. This decrease was greater in the moderately and heavily stained material than in the lightly stained, and more marked after twelve weeks than after six weeks' incubation, indicating that progressive loss in strength accompanies the development of the staining fungus in the wood. The decrease was more pronounced in the unsterilized than in the sterilized material.

In similar tests on naturally stained Scots pine no significant reduction in this property was found as a result of staining.

(3) *Modulus of Elasticity.* The relationship of the modulus of elasticity (stiffness) was erratic. The 9 per cent decrease obtained after six weeks' incubation of the unsterilized material was found to be statistically significant while the 5 per cent decrease after twelve weeks' incubation was not significant. A combination of the results gave an average decrease of about 7 per cent which was also found to be significant. It may be concluded that there is therefore a real decrease in stiffness for the unsterilized material, though this is not of a high order. The decrease for the sterilized material was of a much smaller order and not significant.

Similar tests on Scots pine (2) showed that, in the case of unsterilized timber, a slight degree of blue stain caused no reduction in the modulus of elasticity, but heavy staining was accompanied by a decrease which, though significant, was not sufficiently large to be of practical importance.

(4) *Toughness.* In all cases a marked decrease in toughness accompanied the staining, whether light or heavy. The smallest percentage decrease shown for any group, viz., 16.6 per cent for the lightly stained unsterilized material after six weeks' incubation, was found to be significant, and it may therefore be assumed that the other greater differences are also significant.

In every case, the more heavily stained material showed a greater decrease in toughness than the lightly stained, and this decrease in the case of the unsterilized material was much more marked after twelve than after six weeks' incubation, though this was not true in the case of the sterilized material.

The decreases in toughness for the unsterilized timber do not differ greatly from those for the sterilized. This is in marked contrast to the results obtained for equivalent fibre stress at maximum load in which the decrease for unsterilized timber was markedly greater than that for the sterilized. The comparison is brought out clearly in the following table in which the results for six weeks and twelve weeks' incubation have been combined :

	Per cent decrease.	
	Toughness.	Equivalent fibre stress at max. load.
Unsterilized—Lightly stained	21.7	6.5
More heavily stained	31.4	16.4
Sterilized —Lightly stained	19.6	1.0
More heavily stained	35.2	9.1

It would appear that the steam treatment used in sterilizing the test pieces had no effect in rendering the timber more liable to attack by *Botryodiplodia*. In the previous tests on pine it was found that an appreciably greater reduction in toughness occurred in sapwood which had been steamed before exposure to the staining fungi than in normal unsteamed wood.

Chemical Analysis.

As it was found that this fungus caused an appreciable reduction in bending strength as well as a significant reduction in nominal specific gravity, it was suspected that some attack on the cell wall substance was taking place in addition to depletion of the cell contents. A chemical analysis of the heavily stained sterilized samples and of the matched unstained but steamed control pieces was undertaken by Mr. D. F. Packman in the Chemical Section of this Laboratory. The samples were reduced to sawdust which was sifted to a 60-80 mesh dust. The analyses were carried out by the standard laboratory methods. The following results were obtained. Note that the figures for the infected wood are based on the original weight of the samples :

Table III.

Effect of *Botryodiplodia theobromae* on Obeche Wood.

	Original wood Steam-sterilized. Per cent.	Infected wood (sterilized before infection). Per cent.
Loss in dry weight (from change in specific gravity)	—	12.5
Hot water soluble	7.09	2.70
Alkali „	17.19	10.91
Cellulose content	44.64	41.30
Pentosan „	19.36	16.96
Pentosan in cellulose	6.96	6.65
Pentosan *not* in cellulose	12.4	10.31
Lignin content	33.00	30.94
Methoxyl	6.38	5.83
Methoxyl in lignin	5.94	5.38

It will be seen from the above table that in addition to causing a considerable depletion in the water soluble constituents of the wood (i.e. cell contents and reserve food materials) the fungus has attacked the cellulose

and lignin. This explains why it is able to bring about a reduction in the bending strength.

The decay appears to be typical of an early stage of a white rot in which the alkali solubility is reduced, while at the same time the cellulose, lignin and pentosans are attacked. It has been rather generally assumed that all sap staining fungi derive their nourishment almost entirely from the reserve food materials in the medullary rays. While this may be true for the fungi which cause blue stain in coniferous woods, these results show that other so-called "staining" fungi may in fact cause slight incipient decay which renders the wood weaker in addition to discolouring it.

SUMMARY.

1. A series of mechanical tests were carried out on small selected samples of Obeche cut from a freshly imported log. One set of tests was made on samples which were exposed under controlled conditions to infection with *Botryodiplodia theobromae*, a fungus which frequently causes staining of this timber in the log, and the other set of tests was carried out on carefully matched control samples. In each case one-half of the samples were sterilized by intermittent steaming.
2. Infection with the fungus brings about (a) a marked reduction in toughness amounting in one group of heavily stained specimens to 43 per cent, (b) a definite reduction in bending strength which in one group amounted to 19.7 per cent, (c) a reduction of about 7 per cent in stiffness in the unsterilized timber, and a negligible change in the sterilized timber, (d) a reduction in specific gravity—the maximum loss found amounting to 12.5 per cent.
3. Chemical analysis of the heavily stained samples shows that the fungus causes a marked depletion in the content of water soluble materials, and that it also attacks the cellulose, pentosans and lignin, behaving like a typical white rot fungus.

ACKNOWLEDGMENTS.

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